Appl. No.	:	10/764,978
Confirmation No.	:	9303
Applicant	:	Plamen Denchev
Filed	:	January 23, 2004
Title	:	METHODS FOR REPRODUCING CONIFERS BY SOMATIC EMBRYOGENESIS
TC/A.U.	:	1661
Examiner	:	Hwu, June
Docket No.	:	205502-9037-US00

I, Sally Sorensen, hereby certify that this correspondence is being electronically filed with the United States Patent and Trademark Office on the date of my signature.

Sally Sorensen
Signature

May 16, 2008
Date of Signature

DECLARATION OF LARRY FOWKE
UNDER 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Larry Fowke, do hereby declare and state the following:

1. I am currently an Emeritus Professor in the Department of Biology at the University of Saskatchewan.

2. I received a Bachelors of Arts Degree in Biology from the University of Saskatchewan in 1963. I received a Ph.D. in Plant Cell Biology from the Carleton University in 1968.

3. Attached hereto as Exhibit A is a copy of my Curriculum Vitae.

4. I have read and understand the invention as disclosed in the above-identified patent application, including the invention described by the presently pending claims.

5. I have reviewed the Office Action of January 16, 2008. I understand that claims 1, 5-9, 12-13, 16-23, 27, 28, 33-34, and 36-43 are rejected under 35 U.S.C. § 103(a) as unpatentable over Attree (U.S. Patent No. 6,627,441) in view of Handley (U.S. Patent No. 5,491,090). I also understand that claims 50-54 are rejected under 35 U.S.C. § 103(a) as unpatentable over Fan (U.S. Patent No. 6,689,609) in view of Handley. I also

understand that claims 55-60 are rejected under 35 U.S.C. § 103(a) as unpatentable over Coke (U.S. Patent No. 5,534,433) in view of Pullman (U.S. Patent No. 6,492,174). I believe that the evidence presented herein demonstrates that the pending claims are not obvious in light of the cited references.

6. Embryogenesis *in vitro* is a multi-step process. First, the embryonic cultures must be induced from starting explants in an induction medium in which somatic tissue from the plant must de-differentiate and begin proliferating. The resultant cultures can be maintained, and in somatic embryogenesis reproduced, in a maintenance or proliferation medium. During this step the main goal is for the cells to proliferate as fast as possible, without developing, while maintaining the ability to mature, germinate and produce seedlings. The media used during induction and maintenance contain a relatively high level of hormones, such as auxin and cytokinin, which allow the cells to proliferate, but not differentiate. The next step, prematuration, is optional and is meant to provide a gentle transition from maintenance, where the cells proliferate, but do not differentiate, to maturation, where proliferation slows and differentiation of the cells is encouraged. The prematuration medium contains less auxin and cytokinin than the maintenance medium. The prematuration medium may also contain ABA and may have increased water stress as compared to the maintenance medium. The embryonic cultures must then be stimulated to undergo maturation by incubation in a maturation or development medium. The maturation medium does not contain any auxin or cytokinin. During maturation, the cells stop proliferating, and begin differentiating into embryos. The mature embryos can then be germinated to produce somatic seedlings. Each step in the process is defined by the media used and the effects of the media on the cells.

7. The media and culture conditions used in each of the above described steps are distinct. In particular, maturation media do not contain any auxin or cytokinin to stimulate cell growth. The induction and maintenance media contain auxin or cytokinin to stimulate cellular proliferation. The prematuration media have less auxin and cytokinin than the induction or maintenance media. In addition, maturation media have a high osmoticum and ABA added to stimulate differentiation of the cells, while induction and maintenance media have low osmoticum and no ABA, both of which are important to stimulate

cellular differentiation. Prematuration media provide a gentle transition to maturation media. Prematuration media contain some ABA and have a higher osmoticum than the induction and maintenance media.

8. The pending claims are drawn to methods for reproducing coniferous somatic embryos by somatic embryogenesis comprising growing an embryogenic culture derived from an explant on a nutrient medium comprising lactose, lactose and an additional sugar or a galactose-containing sugar and an additional sugar in steps prior to the maturation step.

9. As stated above, induction, maintenance and prematuration are steps prior to maturation and the media used during these steps help the conifer cells to remain undifferentiated and to proliferate. Maturation requires the cells to slow or stop proliferating and differentiate. Germination requires further differentiation to produce seedlings. Because the goals at the different steps of the process are exactly opposite, the media used at different stages of the method are, and would be expected to be, distinct.

10. Prior to the present application, lactose was not believed to be metabolized by conifer cells and non-metabolizable sugars would not normally be added to the induction, maintenance or prematuration media. The results presented in Example 5 of the pending application demonstrate that lactose and galactose are utilized by conifer cells. It is unexpected that lactose and galactose, sugars not generally available to plants, are metabolized by conifer cells.

11. In addition, the results presented in the Examples of the present application demonstrate that addition of galactose-containing sugars, and lactose in particular, to the induction, maintenance and/or prematuration media increased the number of somatic embryos produced per gram of tissue as compared to cells grown in the presence of other more commonly used sugars such as sucrose or maltose. It was surprising that a galactose-containing sugar could be used at all during induction, maintenance and prematuration, and even more unexpected that use of a galactose-containing sugar would produce superior results as compared to sucrose and maltose.

12. For the reasons set forth above in paragraphs 6-11, the results demonstrated in the Examples section of the present application are surprising and would not be expected based on the cited references.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: May 15, 2008

Larry Fowke, Ph.D.

Docket No.: 205502-9037-US00
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CURRICULUM VITAE FOR LARRY FOWKE

- July 2003-

1. PERSONAL

Born June 6, 1941
Married - 3 children

2. ACADEMIC CREDENTIALS

Earned D.Sc., University of Saskatchewan, 2006
Ph.D. Carleton University, 1968, Biology Department, Plant Cell Biology
B.A. Honours, University of Saskatchewan, 1963, Biology Department

3. APPOINTMENTS AND PROMOTIONS - BIOLOGY, UNIVERSITY OF SASKATCHEWAN

Emeritus Professor, 2006-present
Professor, 1979- 2006
Head of Department, 1994-2000
Assistant Head of Department, 1992-1994
Associate Professor, 1974-1979
Assistant Professor, 1970-1974

4. SPECIAL AWARDS

ISI HighlyCited Researcher for period 1981 - 1999 (Plant and Animal Category)
Jarislowsky Chair in Biotechnology, 2002-2003.
Rawson Professor of Biology, 3 year appointment from July 2002.
Finalist (twice) for Annual Innovation Place/UST Award of Innovation, (2002 - 2005).
Distinguished Researcher Award, University of Saskatchewan, Spring 1998.
NSERC PDF, 1968-1970.

5. TEACHING

Nominated three times for University of Saskatchewan Students Union Teaching Excellence Award.

Regular teaching duties: participated in courses in general biology, cell biology, plant anatomy, plant development, electron microscope techniques.

6. INVITED SYMPOSIUM TALKS AND PLENARY LECTURES

Symposium talk, NATO Advanced Research Workshop, Kiev, Ukraine, 2002.
Symposium talk, International Association of Plant Tissue Culture & Biotechnology, Florida, USA, 2002
Symposium talk, International Union of Forest Research Organizations, Quebec, Canada, 1997.
Symposium talk, Can. Soc. Plant Physiol./Can. Bot. Assoc., Guelph, Canada, 1995.
Symposium talk, Congress on Cell and Tissue Culture, Raleigh, U.S.A., 1994.
Symposium talk, XV International Botanical Congress, Yokohama, Japan, 1993.
Symposium talk, 1st Asia-Pacific Conference on Plant Cell and Tissue Culture, Taejon, Korea, 1993.
Symposium talk, Plant Cell Biotechnologies Conference, Sofia, Bulgaria, 1993.
Symposium talk, Vesicle Traffic in Plants Conference, Gottingen, Germany, 1992.
Plenary Lecture, 8th International Protoplast Symposium, Uppsala, Sweden, 1991.

Symposium talk, Society for Experimental Botany, Warwick, England, 1990.
Symposium talk, Gordon Research Conference on Plant Cell and Tissue Culture, Plymouth, U.S.A., 1989.
Keynote talk, 4th International Conifer Tissue Culture Work Group, Saskatoon, Canada, 1988.
Symposium talk, Interkingdom Workshop on Membrane Traffic and Recycling in Eukaryotes, Wesley Chapel, Florida, 1988.
Symposium talk, XIV International Botanical Congress, Berlin, Germany, 1987.
Symposium talk, 7th International Protoplast Symposium, Wageningen, The Netherlands, 1987.
Symposium talk, NATO Advanced Study Institute, Albufeira, Portugal, 1987.
Plenary Lecture, VI International Congress of Plant Tissue and Cell Culture, Minneapolis, USA, 1986.
Symposium talk, British Society for Cell Biology, Norwich, England, 1986.
Symposium talk, Scandinavian Electron Microscope Society, Copenhagen, Denmark, 1984.
Symposium talk, 6th International Protoplast Symposium, Basel, Switzerland, 1983.
Symposium talk, V International Congress of Plant Tissue and Cell Culture, Tokyo, Japan, 1982.
Symposium talk, International Symposium for Plant Cell Culture in Plant Improvement, Calcutta, India, 1981.
Session organizer and symposium talk at XIII International Botanical Congress, Sydney, Australia, 1981.
Symposium talk, American Tissue Culture Association, Seattle, USA, 1979.
Symposium talk, International Congress for Plant Cell and Tissue Culture, Calgary, Canada, 1978.
Symposium talk, 13th Annual Electron Microscope Colloquium, Ames, USA, 1976

7. TRAINING

7 Ph.D. students, 21 postdoctoral fellows and research associates, 10 international visitors and numerous research technicians.

8. PROFESSIONAL PRACTICE

Advisory Board, Protoplasma, 1984-present
Numerous photographs published in books and review articles
NSERC grants selection committee, Cell Biology, 2003-04
Editor, Plant Cell Reports, 1997-2006
Advisory Board, Cell Biology International, 1987- 2004
Advisory Board, Plant Cell Reports, 1981-1984
Co-editor, Pro-Tem, Canadian Journal of Botany, 6 months, 1983
Associate Editor, Canadian Journal of Botany, 1979-1982
Congress Secretary, International Association of Plant Tissue Culture Congress, Calgary, 1978
Canadian Correspondent, International Association of Plant Tissue Culture, 1978-1982
Western Canadian Director, Canadian Society for Cell Biology, 1972-19, 1978-1980
NSERC grants selection committee, Cell Biology and Genetics, 1985-88, Chairman 1987-88

9. PUBLICATIONS

PAPERS IN REFEREED JOURNALS

124. J. Kang, Y. Mizukami, H. Wang, L. Fowke and N.G. Dengler. 2007 Modification of cell proliferation patterns alter leaf vein architecture in *Arabidopsis thaliana*. **Planta** 226: 1207-1218.

123. D. Bird, M. Buruiana, Y. Zhou, L. Fowke and Hong Wang. 2007. *Arabidopsis* cyclin-dependent kinase inhibitors are nuclear-localized and show different localization patterns within the nucleoplasm. **Plant Cell Reports** 26: 861-872.

122. Y. Zhou, H. Niu, F. Brandizzi, L.C. Fowke and H. Wang. 2006. Molecular control of nuclear and

subnuclear targeting of the plant CDK inhibitor ICK1 and ICK1-mediated nuclear transport of CDKA. **Plant Molecular Biology** 62: 261-278.

121. G. Pan, Y. Zhou, S. Gilmer and L.C. Fowke. 2004. An efficient method for flow cytometric analysis of pollen and detection of 2n nuclei in *Brassica napus* pollen. **Plant Cell Reports** 23: 196-202.

120. Y. Zhou, G Li, F Brandizzi, L Fowke and H Wang. 2003. The plant cyclin-dependent kinase inhibitor ICK1 has distinct functional domains for in vivo kinase inhibition, protein stability and nuclear localization. **The Plant Journal** 35: 476-489.

119. Y. Zhou, H. Wang, S. Gilmer, S. Whitwill and L.C. Fowke. 2003. Effects of co-expressing plant CDK inhibitor ICK1 and D-type cyclin genes on plant growth, cell size and ploidy in *Arabidopsis thaliana*. **Planta** 216: 604-613..

118. Y. Zhou, H. Wang and L.C. Fowke. 2002. Control of petal and pollen development by the plant cyclin-dependent kinase inhibitor ICK1 in transgenic Brassica plants. **Planta** 215: 248-257.

117. Y. Zhou, L.C. Fowke and H. Wang. 2002. Plant CDK inhibitors: studies of interactions with cell cycle regulators in the yeast two-hybrid system and functional comparisons in transgenic *Arabidopsis* plants. **Plant Cell Reports** 20: 967-975.

116. A.L Cleary, L.C. Fowke, H. Wang and P.C.L. John. 2002. The effect of ICK1, a plant cyclin-dependent kinase inhibitor, on mitosis in living plant cells. **Plant Cell Reports** 20: 814-820.

115. H. Wang, Y. Zhou, S. Gilmer, S. Whitwell and L.C. Fowke. 2000. Expression of the plant cyclin-dependent kinase inhibitor ICK1 affects cell division, plant growth and morphology. **The Plant Journal** 24: 613-623.

114. J.D.I. Harper, L.C. Fowke, S. Gilmer, R.L. Overall and J. Marc. 2000. A centrin homologue is localized across the developing cell plate in gymnosperms and angiosperms. **Protoplasma** 211:207-216.

113. H. Lui, H. Wang, C. DeLong, L.C. Fowke, W.L. Crosby and P.R. Fobert. 2000. The *Arabidopsis* Cdc-2a-interacting protein ICK2 is structurally related to ICK1 and is a potent inhibitor of cyclin-dependent kinase activity in vitro. **The Plant Journal** 21: 379-385.

112. L.C. Fowke, T. Dibbayawan, O. Schwartz, J. Harper and R. Overall. 1999. Combined immunofluorescence and field emission scanning electron microscope study of plasma membrane-associated organelles in highly vacuolated suspensor cells of white spruce somatic embryos. **Cell Biol. Internat.** 23: 389-397.

111. S. Gilmer, P. Clay, T.H. MacRae and L.C. Fowke. 1999. Tyrosinated, but not detyrosinated, α-tubulin is present in root tip cells. **Protoplasma** 210: 92-98.

110. D.A. Reid, J.N.A. Lott, S.M. Attree and L.C. Fowke. 1999. Imbibition of white spruce seeds and somatic embryos: a study of morphological changes in an environmental scanning electron microscope and potassium leakage. **In Vitro Cell. Dev. Biol. - Plant.** 35: 303-308.

109. D.A. Reid, J.N.A. Lott, S.M. Attree and L.C. Fowke. 1999. Mineral nutrition in white spruce (*Picea glauca* [Moench] Voss) seeds and somatic embryos. II. EDX analysis of globoids and Fe-rich particles. **Plant Science** 141: 19-27.

108. D.A. Reid, J.N.A. Lott, S.M. Attree and L.C. Fowke. 1999. Mineral nutrition in white spruce (*Picea glauca*

[Moench] Voss) seeds and somatic embryos. I. Phosphorus, phytic acid, potassium, magnesium, calcium, iron and zinc. **Plant Science** 141: 11-18.

107. S. Gilmer, P. Clay, T.H. MacRae and L.C. Fowke. 1999. Acetylated tubulin is found in all microtubule arrays of two species of pine. **Protoplasma**. 207: 174-185.

106. L. Kong, S.M. Attree and L.C. Fowke. 1998. Effects of polyethylene glycol and methylglyoxal bis (guanylhydrazone) on endogenous polyamine levels and somatic embryo maturation in white spruce (*Picea glauca*). **Plant Science** 133: 211-220.

105. H. Wang, Q. Qui, P. Shorr, A.J. Cutler, W.L. Crosby and L.C. Fowke. 1998. ICK1, a cyclin-dependent protein kinase inhibitor from *Arabidopsis thaliana* interacts with both Cdc2a and cyclin D3 and its expression is induced by abscisic acid. **The Plant Journal** 17: 501-510.

104. K. Ilic-Grubor, S.M. Attree and L.C. Fowke. 1998. Comparative morphological study of zygotic and microspore-derived embryos of *Brassica napus* L. as revealed by scanning electron microscopy. **Ann. Bot.** 82: 157-165.

103. K. Ilic-Grubor, S.M. Attree and L.C. Fowke. 1998. Induction of microspore-derived embryos of *Brassica napus* L. with polyethylene glycol (PEG) as osmoticum in a low sucrose medium. **Plant Cell Reports** 17: 329-333.

102. D. Evans, P.J. Clay, S. Attree and L.C. Fowke. 1997. Visualization of Golgi apparatus in methacrylate embedded conifer embryo tissue using the monoclonal antibody JIM 84. **Cell Biol. Internat.** 21: 295-302.

101. H. Wang, L.C. Fowke and W.L. Crosby. 1997. A cyclin-dependent kinase inhibitor gene from *Arabidopsis thaliana*. **Nature** 386: 451-452.

100. L. Kong, S.M. Attree and L.C. Fowke. 1997. Changes in endogenous hormone levels during seed and zygotic embryo development in *Picea glauca* (Moench) Voss. **Physiol. Plant.** 101: 23-30.

99. P. Binarova, C. Cihalikova, J. Dolezel, S. Gilmer and L.C. Fowke. 1996. Actin distribution in somatic embryos and embryogenic protoplasts of white spruce *(Picea glauca)*. **In Vitro Cell. Dev. Biol - Plant** 32: 59-65.

98. A.R. Davis, L.C. Fowke, V.K. Sawhney and N.H. Low. 1996. Floral nectar secretion and ploidy in *Brassica rapa* and *B. napus* (Brassicaceae). II.

Quantified variability of nectary structure and function in rapid-cycling lines. **Ann. Bot.** 77: 223-234.

97. S.M. Attree, M.K. Pomeroy and L.C. Fowke. 1995. Development of white spruce [*Picea glauca* (Moench.) Voss] somatic embryos during culture with abscissic acid and osmoticum, and their tolerance to drying and frozen storage. **J. Exper. Bot.** 46: 433-439.

96. I. Leal, S. Misra, S.M. Attree and L.C. Fowke. 1995. Effect of abscissic acid, osmoticum and desiccation on 11s storage protein gene expression in somatic embryos of white spruce. **Plant Science** 106: 121-128.

95. A.R. Davis, V.K. Sawhney, L.C. Fowke and N.H. Low. 1994. Floral nectary secretion and ploidy in *Brassica rapa* and *B. napus* (Brassicaceae). I. Nectary size and nectar carbohydrate production and composition. **Apidologie** 25: 602-614.

94. P. Binarova, P. Rennie and L.C. Fowke. 1994. Probing microtubule organizing centres with MPM-2 in dividing cells of higher plants using immunofluorescence and immunogold techniques. **Protoplasma** 180: 106-117.

93. L.C. Fowke, S.M. Attree and P.J. Rennie. 1994. Scanning electron microscopy of hydrated and desiccated mature somatic embryos and zygotic embryos of white spruce (*Picea glauca* [Moench] Voss). **Plant Cell Reports** 13: 612-618.

92. S.M. Attree, M.K. Pomeroy and L.C. Fowke. 1994. Production of vigorous, desiccation tolerant white spruce *(Picea glauca* [Moench] Voss) synthetic seeds in a bioreactor. **Plant Cell Reports** 13: 601-606.

91. S.M. Attree and L.C. Fowke. 1993. Embryogeny of gymnosperms: advances in synthetic seed technology of conifers. **Plant Cell, Tissue and Organ Culture** 35: 1-35.

90. S. Misra, S.M. Attree, I. Leal, L.C. Fowke. 1993. Effect of abscisic acid, osmoticum and desiccation on synthesis of storage proteins during the development of white spruce somatic embryos. **Ann. Bot.** 71: 11-22.

89. M. Galway, P.J. Rennie and L.C. Fowke. 1993. Ultrastructure of the endocytotic pathway in glutaraldehyde-fixed and high-pressure frozen/freeze-substituted protoplasts of white spruce (*Picea glauca*). **J. Cell Sci.** 106: 847-858.

88. L.C. Fowke. 1993. Microtubules in dividing root cells of the conifer *Pinus radiata* and the cycad *Zamia furfuracea*. **Cell Biol. Internat.** 17: 143-151.

87. T. Tautorus, H. Wang, L.C. Fowke, D.I. Dunstan, 1992. Microtubule pattern and the occurrence of pre-prophase bands in embryogenic cultures of black spruce (*Picea mariana* Mill.) and non-embryogenic cultures of jack pine (*Pinus banksiana* Lamb.). **Plant Cell Reports** 11: 419-423.

86. T.I. Baskin, C.H. Busby, L.C. Fowke, M. Sammut, F. Gubler, 1992. Improvements in immunostaining samples embedded in methacrylate: localization of microtubules and other antigens throughout developing organs in plants of diverse taxa. **Planta** 187: 405-413.

85. S.M. Attree, M.K. Pomeroy, L.C. Fowke, 1992. Manipulation of conditions for the culture of somatic embryos of white spruce for improved triacylglycerol biosynthesis and desiccation tolerance. **Planta** 187: 395-404.

84. R.M. Napier, L.C. Fowke, C. Hawes, M. Lewis, H.R.B. Pelham, 1992. Immunological evidence that plants use both HDEL and KDEL for targeting proteins to the endoplasmic reticulum. **J. Cell Science** 102: 261-271.

83. S.M. Attree, D. Moore, V.K. Sawhney, L.C. Fowke, 1991. Enhanced maturation and desiccation tolerance of white spruce (*Picea glauca* [Moench]) somatic embryos. Effects of a non-plasmolysing water stress and abscisic acid. **Ann. Bot.** 68: 519-525.

82. H. Wang, A.J. Cutler, L.C. Fowke, 1991. DNA replication and the development of preprophase bands in soybean protoplast cultures. **Physiol. Plant.** 82: 150-156.

81. H. Wang, A.J. Cutler, L.C. Fowke, 1991. Microtubule organization in cultured soybean and black spruce cells. Interphase-mitosis transition and spindle morphology. **Protoplasma** 162: 46-54.

80. H. Wang, G.P. Slater, L.C. Fowke, M. Saleem, A.J. Cutler, 1991. Comparison of cell wall regeneration on maize protoplasts isolated from leaf tissue and suspension cultured cells. **In Vitro Cell. Dev. Biol.** 27P: 70-76.

79. H. Wang, M. Saleem, L.C. Fowke, A.J. Cutler, 1991. DNA synthesis in maize mesophyll protoplasts in relation to source tissue differentiation. **J. Plant Physiol.** 138: 200-203.

78. S.M. Attree, T.E. Tautorus, D.I. Dunstan, L.C. Fowke, 1990. Somatic embryo maturation, germination, and soil establishment of plants of black and white spruce (*Picea mariana* and *Picea glauca*). **Can. J. Bot.** 68:

2583-2589.

77. L.C. Fowke, S.M. Attree, H. Wang, D. Dunstan 1990. Microtubule organization and cell division in embryogenic protoplast cultures of white spruce (*Picea glauca*). **Protoplasma** 158: 86-94.

76. T.E. Tautorus, L.C. Fowke, D.I. Dunstan. 1990. Comparative studies of protoplast development in jack pine (*Pinus banksiana* Lamb.). **Can. J. Bot.** 68: 1774-1779.

75. H. Wang, A.J. Cutler, M. Saleem, L.C. Fowke. 1990. Treatment of soybean cells with cell wall degrading enzymes inhibits nuclear division but not DNA synthesis. **J. Plant Physiol.** 135: 404-408.

74. T.E. Tautorus, S.M. Attree, L.C. Fowke, D.I. Dunstan. 1990. Somatic embryogenesis from immature and mature zygotic embryos and embryo regeneration from protoplasts in black spruce (*Picea mariana* Mill.). **Plant Science** 67: 115-124.

73. S.M. Attree, D.I. Dunstan, L.C. Fowke. 1989. Plantlet regeneration from embryogenic protoplasts of white spruce (*Picea glauca*). **Bio/Technology** 7: 1060-1062.

72. T.E. Tautorus, F. Bekkaoui, M. Pilon, R.S.S. Datla, W.L. Crosby, L.C. Fowke, D.I. Dunstan. 1989. Factors affecting transient gene expression in electroporated black spruce (*Picea mariana*) and jack pine (*Pinus banksiana*) protoplasts. **Theor. Appl. Genet.** 78: 531-536.

71. S.M. Attree, S. Budimir, L.C. Fowke. 1989. Somatic embryogenesis and plantlet regeneration from cultured shoots and cotyledons from stored seed of black and white spruce (*Picea mariana* and *Picea glauca*). **Can. J. Bot.** 68: 30-34.

70. H. Wang, A.J. Cutler and L.C. Fowke. 1989. Preprophase bands in cultured multinucleate soybean protoplasts. **Protoplasma** 150: 110-116.

69. S.M. Attree, D.I. Dunstan and L.C. Fowke. 1989. Initiation of embryogenic callus and suspension cultures, and improved embryo regeneration from protoplasts of white spruce (*Picea glauca*). **Can. J. Bot.** 67: 1790-1795.

68. H. Wang, A.J. Cutler, M. Saleem and L.C. Fowke. 1989. Immunocytochemical detection of DNA synthesis in plant cells. **J. Plant Physiol.** 135: 15-20.

67. H. Wang, A.J. Cutler, M. Saleem and L.C. Fowke. 1989. Microtubules in maize protoplasts derived from cell suspension cultures: effect of calcium and magnesium ions. **Europ. J. Cell Biol.** 49: 80-86.

66. L.C. Fowke, M.A. Tanchak and P.J. Rennie. 1989. Serial

section analysis of coated pits and coated vesicles in soybean protoplasts. **Cell Biol. Internat. Rep.** 13: 419-425.

65. H. Wang, A.J. Cutler and L.C. Fowke. 1989. High frequencies of preprophase bands in soybean protoplast cultures. **J. Cell Sci.** 92: 575-580.

64. H. Wang, A.J. Cutler, M. Saleem and L.C. Fowke. 1989. Microtubules in maize leaf protoplasts in relation to donor tissue and in vitro culture. **Protoplasma** 150: 48-53.

63. L.R. Griffing, L.C. Fowke and F. Constabel. 1989. Radioimmunoassay of morphinan alkaloids in *Papaver somniferum* hypocotyls, callus, and suspension cultures. **J. Plant Physiol.** 134: 645-650.

62. H. Wang, A.J. Cutler, M. Saleem and L.C. Fowke. 1989. DNA replication in maize leaf protoplasts. **Plant Cell, Organ and Tissue Culture** 48: 33-46.

61. K.M. Thai, S. Jana and L.C. Fowke. 1989. Cell membrane permeability and ultrastructural effects of difenzoquat on wild oats (*Avena fatua*). **Weed Science** 37: 98-106.

60. M.A. Tanchak, P.J. Rennie and L.C. Fowke. 1988. Ultrastructure of the partially coated reticulum and dictyosomes during endocytosis by soybean protoplasts. **Planta** 175: 433-441.

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58. K. Kartha, L.C. Fowke, N.L. Leung, K.L. Caswell and I. Hakman. 1988. Induction of somatic embryos and plantlets from cryopreserved cell cultures of white spruce (*Picea glauca*). **J. Plant Physiol.** 132: 529-539.

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56. I. Hakman, P. Rennie and L. Fowke. 1987. A light and electron microscope study of *Picea glauca* (white spruce) somatic embryos. **Protoplasma** 140: 100-109.

55. M.A. Tanchak and L.C. Fowke. 1987. The morphology of multivesicular bodies in soybean protoplasts and their role in endocytosis. **Protoplasma** 138: 173-182.

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protoplasts from an embryogenic cell suspension culture of *Picea glauca* (Moench) Voss. **Plant Cell Reports** 6: 476-479.

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